CORRELATION BETWEEN TOTAL LYMPHOCYTE COUNT AND CD4 LYMPHOCYTE COUNT IN CHILDREN INFECTED WITH HIV

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CERTIFICATE

Certified that this dissertation entitled **"CORRELATION BETWEEN TOTAL LYMPHOCYTE COUNT AND CD4 LYMPHOCYTE COUNT IN CHILDREN INFECTED WITH HIV"** is a bonafide work done by DR.M.CHEZHIYAN, M.D. Post graduate student of Pediatric Medicine, **INSTITUTE OF CHILD HEALTH AND HOSPITAL FOR CHILDREN, EGMORE, CHENNAI - 600 008,** attached to Madras Medical College, during the academic year 2003-2006.

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DECLARATION

I declare that this dissertation entitled **"CORRELATION BETWEEN TOTAL LYMPHOCYTE COUNT AND CD4 LYMPHOCYTE COUNT IN CHILDREN INFECTED WITH HIV"** has been conducted by me at the Institute of Child Health and Hospital for Children, under the guidance and supervision of my unit chief **Prof. T.K.Vasantha Mallika M.D.,D.C.H.** It is submitted in part of fulfillment of the award of the degree of M.D. (Pediatrics) for the February 2006 examination to be held under the Tamil Nadu Dr.M.G.R Medical University, Chennai. This has not been submitted previously by me for the award of any degree or diploma from any other university.

(Dr.M.CHEZHIYAN)

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CONTENTS

INTRODUCTION

As of the end of 2003, an estimated 42 million people worldwide - 38.6 million adults and 3.2 million children younger than 15 years - were living with HIV/AIDS. Approximately 70 percent of these people (29.4 million) live in Sub-Saharan Africa; another 17 percent (7.2 million) live in Asia [(1).] Worldwide, approximately twelve of every 1000 adults aged 15 to 49 are HIV-infected. In Sub-Saharan Africa, about 9 percent of all adults in this age group are HIV-infected. In 4 African countries, the prevalence of HIV infection among adults aged 15 to 49 exceeds 30 percent [(1)] .Approximately 50 percent of adults living with HIV/AIDS worldwide are women. An estimated 5 million new HIV infections occurred worldwide during 2003; that is, about 14,000 infections each day. More than 95 percent of these new infections occurred in developing countries. In 2003, approximately 2,000 children under the age of 15 years, and 6,000 young people aged 15 to 24 years became infected with HIV every day. In 2003 alone, HIV/AIDS-associated illnesses caused the deaths of approximately 3.1 million people worldwide, including an estimated 610,000 children younger than 15 years.: The first HIV case in India was reported in 1986 in Tamil Nadu. The estimated number of adults living with HIV/AIDS by end of 2003 is 50 lakhs of which children form 1.2 lakhs and women 1.9 lakhs. Indian statistics reveal the high HIV prevalence states in India to be Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Nagaland and Manipur where HIV infection has crossed 1% or more in antenatal women. The national HIV prevalence in adult population is 0.9%.

Pediatric AIDS threatens much of the progress made in child survival in developing countries over the past 10 to 15 years. It is estimated that more than 8 lakhs of new pediatric infections are acquired, world wide, every year mostly through maternal foetal transmission.

THE VIRUS: [22]

HIV types 1 and 2 are members of the retroviridae family and belong to the lenti virus genus. The HIV genome is a single stranded RNA 9.8 kb in size, with identical regions at both ends of the genome that contain important regulatory genes. The remainder of genome includes 3 major coding regions.

The GAG region encodes the viral core proteins. The POL region encodes the viral enzymes. The ENV region encodes the viral envelope proteins gp 120 and gp 41. Other proteins are involved in Transcription (TAT),viral mRNA expression(REV), viral enhanced infectivity (NEF).

The major external viral protein of HIV is gp120, a heavily glycosylated protein associated with the transmembrane glycoprotein gp41. the gp41 is very immunogenic and is used to detect HIV antibodies in diagnostic assays.

Secondary receptors for attachment have also been identified, including the fusion inducing molecule CXCR-4 which is a co-receptor for HIV attachment to lymphocytes, and CCR-5,a β chemokine receptor which facilitates HIV entrance into macrophages.

TRANSMISSION

Transmission of HIV occurs via sexual contact, parenteral exposure to blood, or vertical transmission from mother to child. The primary route of infection in pediatric population is vertical transmission, accounting for virtually all new cases. Reported vertical transmission rates are between 20-40%. Perinatal treatment of HIV infected mothers with anti-retro viral drugs can dramatically reduce rates to less than 8%.

Vertical transmission of HIV [12, 13, 14] can occur before (intrauterine), during (intrapartum), or after delivery (through breast feeding) .Intrauterine transmission[21] can occur as early as 10 weeks of gestation. It is generally accepted that 30-40% of infected newborns are infected in-utero, because this percentage of infants have lab evidence of infection (positive viral culture) within first week of life.

The highest percentage of HIV infected children acquire the virus intrapartum, evidenced by finding that 60-70 % of infected infants do not demonstrate virus before 1 week of life. The mechanism of transmission appears to be exposure to infected blood and cervico vaginal secretions in the birth canal, where HIV is found in high titer during late gestation and delivery.

Several risk factors influence the rate of vertical transmission : preterm delivery, low birth weight, low maternal antenatal CD4+ counts and IV drug use during pregnancy. The single most important variable appears to be more than 4 hr duration of ruptured membranes , which doubles the transmission

rates. Caesarean section may confer a protective effect compared to vaginal delivery.

The risk of transmission of HIV infection to infants by breast feeding [15] appears to be around 14%.WHO recommends that in developing countries the benefits of breast feeding outweighs the risk of HIV transmission, and HIV infected women should breast feed their children.

Transfusions of blood and blood products accounted for about 3-6% of pediatric HIV infections in the past. However this is on the decline now .

Sexual transmission in the pediatric population is infrequent but a small number of cases relating to sexual abuse have been reported.

PATHOGENESIS: [23,24]

In adults and adolescents, after HIV has entered the circulation, intense viremia ensues causing flu like illness in 50-70% of cases. This results in widespread seeding of virus to various organs, including the brain and lymphoid tissues. HIV selectively binds to cells expressing the CD4+ molecules on their surface and cells of monocyte macrophage lineage. HIV may also infect other cells such as microglia,astrocytes,oligodendroglia,and placental tissue containing villous Hofbauer cells.With establishment of a cellular and humoral immune response within 2-4 months,the level of culturable virus from blood declines and patients enter a phase characterized by a lack of symptoms and the return of CD4+ cells to only moderately decresed levels.

Early HIV infection and replication in children have no apparent clinical consequences.Whether tested by virus isolation or by PCR ,less than 50% of HIV –infected infants demonstrate evidence of virus at birth. The virus load increases by 1-4 months and almost all HIV infected infants have demonstrable HIV in peripheral blood by 4 months of age.

Three distinct patterns of disease has been described in children. From 15-25% of HIV infected newborns have a rapid disease course,with onset of symptoms and AIDS during the first few months of life and if untreated , a median survival of 6-9 months[28]. The majority of perinatally infected newborns 60-80% present with a much slower progression of disease,with median survival of 6 years.Many patients in this group have a negative viral culture or PCR test in the first week of life and , are therefore, considered to be infected intrapartum. The viral load increases slowly by 2-3 months of age and then slowly declines over a period of 24 months.

The third pattern of disease involves the long term survivors, which occurs in a small percentage <5% of perinatally infected children who have minimal or no progression of disease with relatively normal levels of CD4+ counts and very low viral loads for more than 8 years.

CLINICAL MANIFESTATIONS: [26]

The clinical manifestations of HIV disease in children differ in important ways from those seen in adults.

1. Overall progression of disease is more rapid.
2. Recurrent invasive bacterial infections more common
3. Disseminated CMV, Candida, Herpes simplex and Varicella zoster infections more common.
4. 20-40% children have encephalopathy
5. Malignancies, neuropathy and myopathy are uncommon.
6. Lymphocytic Interstitial Pneumonitis (LIP) occurs almost exclusively in children.

Bacterial Infections[31]

The incidence of invasive bacterial disease, including meningitis, bacteremia, or pneumonia is higher than among adults. Other bacterial infections, including sinusitis, otitis media, deep tissue abscesses, osteomyelitis,ocular infections[50] and septic arthritis are also more prominent.

Cardiac Involvement

Cardiac disease, including cardiomyopathy, congestive heart failure, and conduction defects is increasingly recognized. Sub clinical myocardial dysfunction is extremely common when routine echocardiography is done. However, in a prospectively studied cohort, the cumulative incidence of overt congestive heart failure was 12% at 2 years. The etiology is poorly understood, but HIV RNA has been isolated from myocardial cells. Left ventricular

dysfunction is correlated with the presence of encephalopathy and is an independent predictor of mortality.

Gastrointestinal Involvement [29]

Gastrointestinal complications of HIV are common in children. Thrush persisting more than 2 months is considered a category B symptom, but other oral manifestations are common. These include candidiasis, linear gingival erythema, herpes stomatitis, necrotizing gingivitis, salivary gland enlargement and aphthous ulcers. Aphthous ulcers are more common in children who are HIV-infected and can involve the esophagus, stomach, rectum, or vulva. Both acute and persistent diarrhea are major causes of morbidity in developed countries. In developing countries, diarrhea is a major cause of death, and, HIV-infected children had an 11-fold increase in risk of death from diarrhea compared to uninfected infants of HIV-infected mothers. The spectrum of diarrheal pathogens is incompletely studied. In general, the pathogens are those common in uninfected children in the region, including *Salmonella*, *Shigella*, *Campylobacter*, rotavirus, and enterotoxigenic, enteroaggregative and locally adherent phenotypes of diarrhea-causing *Escherichia coli*. However, disease is more frequent, more severe, and more likely to be persistent. Cryptosporidiosis and microsporidiosis are late complications.

Pulmonary Involvement

The most important pulmonary complications of HIV in children include bacterial pneumonia, *Pneumocystis carinii* pneumonia[25,39], and

lymphoid interstitial pneumonitis (LIP). The classic reticulonodular findings of LIP may be noted on chest x-ray when the child is asymptomatic and has normal oxygen saturation. As the disease worsens, hypoxia and clubbing may develop. Severe LIP responds to steroids, but the presence of LIP is actually associated with improved survival compared to other manifestations of AIDS.

Renal Involvement[30]

Renal involvement occurs in 2 to 10% of HIV-infected children in the United States. HIV nephropathy can range from mild proteinuria to nephrosis, renal tubular acidosis, hematuria and acute renal failure. In adults in the United States, there is a markedly increased risk of nephropathy among black persons with HIV infection; this appears to be true in children as well but the data are sparse.

Central Nervous System Involvement: [27]

Central nervous system involvement is a common and serious complication of HIV infection in children. Encephalopathy that meets the CDC definition represents the more severe end of the clinical spectrum. Among 1811 children followed in the Pediatric Spectrum of Disease Project, 23% were diagnosed with encephalopathy. In the WITS study of 124 children with a median of 24 weeks of follow-up, 21% of children developed encephalopathy. Milder neurologic dysfunction and developmental difficulties are even more common. A comparison of encephalopathy in adults and children in 2 prospective cohorts found striking differences between children and adults in

early onset encephalopathy, but fewer differences later in disease. The incidence of encephalopathy was 9.9% in children compared to 0.3% in adults in the first year, 4.2% compared to 0% in second year, but about 1% per year thereafter in children as well as adults. Early onset encephalopathy was more severe, was associated with less immunosuppression, and resulted in more dramatic brain atrophy and prominent motor findings in children than in adults. It is hypothesized that the encephalopathy shared by older children and adults occurs by a different process than the devastating early onset encephalopathy of infants. A formal developmental or neurologic assessment should be performed periodically in all infected children.

Malignancies[48]

AIDS-associated malignancies in children differ from those in adults. Non-Hodgkin's lymphoma is the most common malignancy, and GI tract involvement is common. The second most commonly reported malignancy is leiomyosarcoma, a disease that is extraordinarily rare in adults. Kaposi's sarcoma, common among HIV-infected gay men, is very rare in children in developed countries.

Growth & Nutrition

Growth failure is a prominent feature of untreated HIV infection. Stunting, or low height for age, is more prominent than wasting. Growth velocity clearly increases with effective antiretroviral therapy but often does not return to normal. Nutritional assessment is important in all infected children

to maximize growth. It is particularly important in children with advanced disease who may suffer poor appetite, nausea, gastroparesis, increased metabolic demands, diarrhea, or malabsorption. Comprehensive nutritional assessment is useful for all HIV-infected children. Growth hormone has been used to treat persistent growth failure in some children.

Tuberculosis in HIV infection:[18]

Tuberculosis is not only the commonest opportunistic infection, but also the earliest manifestation of HIV.Tuberculosis occurs in HIV infected patients without pre-existing AIDS. This is presumably because of M.tuberculosis being more virulent than other HIV associated pathogens such as P.carinii or M.avium complex. HIV infected children usually develop progressive primary tuberculosis,following recent infection.Clinical and radiological manifestations do not vary significantly from those who are seronegative. Extra pulmonary tuberculosis is common, with lymphadenopathy, pleural effusion and miliary TB being the major types. Majority of TB cases in children are diagnosed clinically without microbiologic confirmation. Tuberculin skin test is more often negative , in children with advanced HIV disease, complicating the issue of diagnosing tuberculosis.

CLINICAL CLASSIFICATION

In the 1994 revised clinical classification of Pediatric HIV infection , children are classified as asymptomatic (Category N), mildly symptomatic with HIV-related symptoms (Category A), moderately symptomatic (Category B)

with significant HIV-related symptoms but no Category C symptoms, and Category C which includes AIDS-defining illnesses as outlined in the 1987 case definition.

In addition to providing a useful standard for evaluating children, the revised classification system has been shown to be useful for establishing prognosis and modeling the course of disease although further modifications have been proposed.

Most children will present for medical care with category A symptoms, but due to the non-specific nature of these symptoms, HIV may not be suspected or diagnosed. Many women with HIV infection do not have easily identified risk factors, so a high index of suspicion is needed. Hepatomegaly and lymphadenopathy are the most common category A findings. Parotitis is less common but more striking. The histologic findings demonstrate a dense infiltrate of CD8 cells within the gland. Cystic degeneration may be seen, and lymphoma within the parotid may develop in rare cases. The triad of lymphadenopathy, splenomegaly, and failure to thrive is strongly suggestive of HIV infection, and testing should be performed.

Category B symptoms indicate more severe disease. Based on Markov modeling, children spend the longest time in Category B, a mean of 65 months compared to 10 months in Category A and 35 months in Category C

Table 1: 1994 Revised human immunodeficiency virus pediatric classification system: Immune categories based on age-specific CD4 + T lymphocyte and percentage.[20]

Category	<12 months		1-5 years		6-12 years	
	No. / microliter	(%)	No. / microliter	(%)	No. / microliter	(%)
Category 1 No suppression	>1,500	(>25%)	>1,000	(>25%)	>500	(>25 %)
Category 2 Moderate suppression	750-1,499	(15%-24%)	500-999	(15%-24%)	200-499	(15%-24%)
Category 3 Severe suppression	<750	(<15%)	<500	(<15%)	<200	(<15%)

Table 2: Revised Human Immunodeficiency Virus pediatric classification system: Clinical categories[20]

Category N: Not Symptomatic

Children who have no signs or symptoms considered to be the result of HIV infection or who have only one of the conditions listed in category A.

Category A: Mildly Symptomatic

Children with two or more of the following conditions but none of the conditions listed in categories B and C:

- Lymphadenopathy (>0.5 cm at more than two sites; bilateral = one site)
- Hepatomegaly

- Splenomegaly
- Dermatitis
- Parotitis
- Recurrent or persistent upper respiratory infection, sinusitis, or otitis media

Category B: Moderately Symptomatic

Children who have symptomatic conditions other than those listed for category A or category C that are attributed to HIV infection. Examples of conditions in clinical category B include but are not limited to the following:

- Anemia (<8gm/dL), neutropenia (<1,000/mm^3), or thrombocytopenia (<100,000/cu mm) persisting >30 days
- Bacterial meningitis, pneumonia, or sepsis (single episode)
- Candidiasis, oropharyngeal (i.e., thrush) persisting for >2 months in children aged >6 months
- Cardiomyopathy
- Cytomegalovirus infection with onset before age 1 month
- Diarrhea, recurrent or chronic
- Hepatitis
- Herpes simplex virus (HSV) stomatitis, recurrent (i.e., more than two episodes within 1 year)

- HSV bronchitis, pneumonitis, or esophagitis with onset before age 1 month
- Herpes zoster (i.e., shingles) involving at least two distinct episodes or more than one dermatome
- Leiomyosarcoma
- Lymphoid interstitial pneumonia (LIP) or pulmonary lymphoid hyperplasia complex
- Nephropathy
- Nocardiosis
- Fever lasting >1 month
- Toxoplasmosis with onset before age 1 month
- Varicella, disseminated (i.e., complicated chickenpox)

Category C: Severely Symptomatic

Children who have any condition listed in the 1987 surveillance case definition for acquired immunodeficiency syndrome, with the exception of LIP (which is a category B condition).

Serious bacterial infections, multiple or recurrent

Candidiasis,esophageal or pulmonary

Coccidioidomycosis,disseminated

Cryptococcosis,Extra-pulmonary

Cryptosporidiasis or isosporiasis with diarrhea persisting for more than 1 month

CMV disease with onset of disease after 1 month of age.

Encephalopathy (atleast 1 of the following present for atleast 2 months in the absence of concurrent illness other than HIV)

Failure to attain or loss of developmental milestones or loss of intellectual ability

a. Impaired brain growth or acquired microcephaly as demonstrated by CT or MRI

b. Acquired symmetric motor deficit manifested by 2 or more of following: paresis,pathologic reflexes,ataxia,gait disturbances

c. HSV infection

d. Histoplasmosis,disseminated

e. Kaposi's sarcoma

f. Primary brain lymphoma

g. Burkitt’s or immunoblastic lymphoma

h. Mycobacterium tuberculosis,Disseminated or Extra pulmonary

i. Mycobacterium avium complex, disseminated

k. Disseminated other or unspecified mycobacterium

l. Pneumocystis carinii pneumonia

m. Progressive multifocal leukoencephalopathy

n. Salmonella septicemia recurrent(non typhoid)

Toxoplasmosis Brain after 1 month age.

Wasting syndrome is defined as wasting in the absence of a concurrent illness other than HIV infection that could explain:

1. Persistent weight loss >10% of baseline

OR

downward crossing of at least two of the following percentile lines on the weight-for-age chart (95th, 75th, 50th, 25th, 5th) in a child >=1 year of age OR

1. <5th percentile on weight-for-height chart on two consecutive measurements >30 days apart

PLUS EITHER

chronic diarrhea (i.e., at least two loose stools per day for >30 days)

OR

- documented fever (for >30 days, intermittent or constant).

WHO has devised a **staging system for HIV infection and disease in children** based on the fifth international WHO Workshop on clinical management of HIV AIDS on 14th October 2003.

Category 1.

Asymptomatic

Generalized lymphadenopathy

Category 2.

Unexplained chronic diarrhea (> 1month)

Severe persistent or recurrent candidiasis

Weight loss or failure to thrive

Persistent fever(> 1month)

Recurrent severe bacterial infections

Category 3

AIDS defining opportunistic infections

Severe failure to thrive

Progressive encephalopathy

Malignancy

Recurrent septicemia or meningitis

Clinical case definition for AIDS in children:

1. Two positive test for HIV infection (by ELISA,Rapid tests)in children older than 18 months or confirmed maternal infection for children younger than 18 months AND

2. Presence of atleast 2 major and 2 minor signs in the absence of a known cause of immunosuppression

Major signs:

a. loss of weight or failure to thrive , not due to any other cause
b. chronic diarrhea for over 1 month
c. prolonged fever for over 1 month

Minor signs:

a. repeated common infections
b. generalized lymphadenopathy
c. oropharyngeal candidiasis
d. persistent cough for over a month
e. disseminated maculopapular dermatosis

PREVENTING MOTHER TO CHILD TRANSMISSION[5,6,7]

A growing number of studies demonstrate the efficacy of various perinatal antiretroviral regimens for preventing transmission of HIV from mothers to babies. Taken together, these studies show that the rate of transmission to the infant, which in untreated HIV-infected pregnant women exceeds 20%, can be dramatically reduced by antiretroviral therapy given in the perinatal period. Regimens found to be effective include treatment of the mother in the third trimester of pregnancy and during labor, with subsequent treatment of the infant for at least the first few days of life. Regimens begun at the time of labor are effective (although somewhat less so) only if followed by treatment of the infant, probably for several weeks. When prenatal care is lacking, treatment of the infant alone, especially if done within the first 48 hours of life, also provides a reduction in transmission risk. The benefit of perinatal treatment is diminished in breastfed infants compared with those who are not breastfed; perinatal treatment in the setting of breastfeeding may serve largely to shift part of the risk of infection from the peripartum period to several months later. The risk of maternal-fetal transmission decreases with decreasing viral load, and is very low in women receiving effective antiretroviral therapy as a matter of routine care.The risk of transmission may reach less than 1% in women with plasma HIV viral load <400 HIV RNA copies/mL on treatment, which is considerably lower than the transmission risks associated with older perinatal regimens . It should therefore be kept in mind that these regimens are only interim measures. The true benefit of treatment will be realized not simply when the rate of infant infection is reduced, but when the health of the mother is preserved as well.

DIAGNOSIS: [32]

Kinetics of antibody response in an HIV infected person.

One should have knowledge about antibody response in HIV infected person for optimum use of different tests during different stages of disease.

1. Viral entry in to body leads to transient period of high plasma viremia & antigenemia. The levels come down with 1-2 months with concomitant immune response.

2. Antibodies are produced against Structural proteins, regulatory proteins,accesory protein. structural protein are strongly immunogenic and almost all tests are based on antibody detection against them gag protein are the first to appear and first to decline. However antibody to env protein persist throughout.

BASICALLY 3 TYPE OF TESTS

1) Anti HIV antibody tests
2) Virological tests
3) Surrogate markers

SPECIMEN USED

Antibody detection	Blood, serum, plasma
Antigen detection	Serum,plasma,csf cell culture supernatant
Viral isolation & detection of viral nucliec acid	Plasma, serum, csf , vaginal & cervical secretions

ANTI .HIV ANTIBODY TEST

1)	Screening test	-	ELISA Rapid test
2)	Supplementaltest	-	Western blot ,line immunoassay ,radio immunoblot assay

ELISA

Most commonly performed test to detect HIV antibody

ELISA tests are scored as positive (reactive), indeterminate(partially reactive)& negative (nonreactive). It has got a high sensitivity of approximately 99.5% , but it is not that specific.

False positive tests occur in no of situation & these includes.

- Acute viral infection
- Recent influencza vaccination
- hepatic disease
- Auto antibodies

Elisa becomes positive 1-2 months after primary infection . Elisa can take up 3 hours to yield result, but it. has the major advantage of being economical. (Single elisa cost you only RS 200)

RAPID TEST

They give results within minutes (3–30 minute)

Most have senstivities & specifities 98% & 99% respectively

Advantages

- quicker to perform and results are delivered on the same day
- Do not require batching
- Do not require specialised equipment or trained personnel

The only disadvantage with rapid test is that it is expensive and according to WHO guidelines ,An individual reactive to three different system of ELISA /rapid tests can be to be said have HIV infection.

WESTERN BLOT

Western blot is the supplementary test to confirm the result of a reactive sample in developed countries, whereas ELISA / rapid tests are also used to confirm the results of reactive samples in developing countries . This is because Western blot & other supplemental test are more expensive & rather difficult to interpret , can also have a minimum level of false positive reactions

Western blot detects different antibodies within the test serum, capable of reacting with different Viral proteins. It is done by electrophoresis of plasma on pre impregnated strip containing various HIV antigens. Western blot in is reported as positive if at least 2 out of 3 bands are positive (P24, GP41, GP120/160).

OTHERS SUPPLEMENTAL TESTS

1) Line immunoassay
2) Radio immunoblot assay(RIBA)

Patient who give indeterminate results need to be retested 8-12 weeks later. Consistent indeterminate results need to be confirmed by virological test.

VIROLOGICAL TEST

Virological test will be needed in the following situations.

For the diagnosis of

1) HIV infection in the newborn
2) HIV infection during the window period
3) HIV infection in persons giving indeterminate WB/LIA/RIBA result
4) Monitor viral load during therapy.
5) Research activities.

HIV P24 antigen

HIV P24 antigen is detected and quantified using EIA technique. It has got sensitivity and specificity of about 77% and 95% respectively. It is undetectable in asymptomatic patients and in infants as it is present as immune complex with anti P24 antibody. The presence and detection of P24 antigen indicates high level of viremia and increased risk of clinical progression.

Immune complex dissociated P24 antigen has improved sensitivity even then the sensitivity level is sub optimal for early diagnosis of HIV in infants.

NUCLIEC ACID DETECTION

1) HIV DNA PCR (Qualitative assay)

This detects the proviral DNA that has integrated with host cell (Peripheral blood mononuclear cells). It has got high sensitivity (96-95%) and specificity (97-98%).

HIV DNA PCR is

- Used for early diagnosis of HIV infection in neonate
- to resolve indeterminate serological tests,

HIV RNA ASSAYS

1) They are primarily used monitor disease progression and response to antiretroviral therapy.

2) HIV RNA analysis is used when there is high degree of viremia and serological test are negative. It has got 100% sensitivity and with few false positives, specificity's may range from 97-98%. Because of the false positive reactions the use of these assay for the diagnosis of HIV Infection is controversial.

VARIOUS ASSAYS

1) RT – PCR

2) NASBA – nucleic Acid sequence based amplifications

3) 'b' DNA technique

The lower level of quantification of HIV RNA by these method range between 50 to 80 copies / ml .Viral load should be undetectable within 3-6 months of therapy, failure to do so indicates that the drugs are not effective.

VIRAL CULTURE

This provides the most direct evidence of HIV infection and it requires high degree of expertise. Autologous/ Heterologous PBMNC activated with mitogens are co-cultivated with infectious material for about 28 days .The presence of virus is detected by the presence of P24 antigen / RT enzyme in culture supernatant.

It is rarely used to make diagnosis and it is mainly used for research purpose.

SURROGATE MARKERS

1. CD4 / CD8 COUNTS
2. Neopterin
3. Beta 2 microglobulin
4. HIV P24 antigen

CD4 COUNTS

CD4 cells are a type of lymphocyte (white blood cell) which are important part of the immune system. .Researchers can tell these cells apart by specific proteins on the cell surface. A T-4 cell is a T-cell with CD4 molecules on its surface. This type of T-cell is also called “CD4 positive,” or CD4+.

WHY ARE CD4 CELLS IMPORTANT IN HIV?

When HIV infects humans, the cells it infects most often are CD4 cells. The virus becomes part of the cells, and when they multiply to fight an infection, they also make more copies of HIV.When someone is infected with HIV for a long time, the number of CD4 cells they have (their CD4 cell count) goes down. This is a sign that the immune system is being weakened. The lower the CD4 cell count, the more likely the person will get sick.

There are millions of different families of CD4 cells. Each family is designed to fight a specific type of germ. When HIV reduces the number of CD4 cells, some of these families can be totally wiped out,one can lose the

ability to fight off the particular germs those families were designed for. If this happens, they might develop an opportunistic infection .

DIAGNOSIS OF HIV INFECTION IN THE NEW BORN

Early diagnosis of HIV infection in infants is essential to institute ART & prophlylactic therapies and at the same time minimize the potential toxicity of these therapies in exposed uninfected children.

The conventional HIV antibody test cannot be used to make diagnosis of HIV in the newborn as maternal antibodies are can be detected even in uninfected newborn for up to a period of 18 months

Test used for early diagnosis of Congenital HIV infections .

1. Detection of IgA and / IgM anti HIV antibodies

IgA antibodies appear by 3-4 months of age and IgM by 6 months of age. IgA after 3 months has a sensitivity of about 97.6% and specificity of about 99.7% .IgM production is erratic and gives false positive results. However serological markers in the child are not reliable due to the transient presence of these markers. Hence virological test are used for the diagnosis of HIV infection in the newborn.

Current practice is to test infants born to HIV infected women at the ages of

48 hours

1 –2 months

3-6 months.

A positive virological at any age suggest the possibility of HIV infection and should be confirmed by second test as soon as possible. However a negative ELISA done between 6 –18 months in the absence of clinical disease rules out HIV infection. If only one PCR can be done due to cost constraints it is best performed between 3- 6 months of age. Cord blood should not be use for HIV testing as there can be contamination.

MANAGEMENT OF HIV INFECTED CHILDREN: [2]

The advent of many powerful antiretroviral agents and recognition of common diseases that attack the children along with powerful monitoring tools, has helped ensure longevity and improve quality of life in the HIV affected children. Although the pathogenesis of human immunodeficiency virus (HIV) infection and the general virologic and immunologic principles underlying the use of antiretroviral therapy are similar for all HIV-infected persons, there are unique considerations needed for HIV-infected infants, children, and adolescents, including a) acquisition of infection through perinatal exposure for many infected children; b) differences in diagnostic evaluation in perinatal infection; c) differences in immunologic markers (i.e., CD4+ T cell count) in young children;[30] d) changes in pharmacokinetic parameters with age caused by the continuing development and maturation of organ systems involved in

drug metabolism and clearance; e) differences in the clinical and virologic manifestations of perinatal HIV infection secondary to the occurrence of primary infection in growing, immunologically immature persons.

For making rational decisions in treatment of HIV infected children , a proper knowledge of the antiretroviral agents, their indications, situations in which they may be changed, other drugs that prevent opportunistic infections and others for supportive care, is a must. In addition we must have an idea of how to respond to various clinical scenarios in HIV infected pregnant mothers in order to prevent HIV in their children as well as about vaccination schedules in the children.

ANTI RETRO VIRAL THERAPY: [4,9.,10]

Right from 1986 when Zidovudine ushered in the era of antiretroviral therapy, there has been an explosion in research and introduction of drugs for the treatment of HIV.

Despite the numerous drugs available the harsh truth is that HIV is not curable – with any form of therapy. Anti-retro viral therapy only prolongs life. It may delay the onset of clinical disease and in case the disease is already symptomatic reduce the morbidity.

In resource poor country like ours – India, where cost is the most important factor limiting therapy, the process of choosing appropriate drug has been simplified with the introduction of several regimens by the WHO.

These regimens were selected on basis of – low toxicity, efficacy and cost effective.

On behalf of GOI, Union ministry of health and family welfare has started a programme for providing ART from 1st of April 2004 free of cost to HIV patients. This will concentrate on the following groups of people.

a. Seropositive mothers under PPTCT programme
b. Seropositive children< 15 yrs
c. HIV patients seeking therapy from govt. hospitals

The goals of ART are

1. Reduction of HIV related morbidity
2. Improvement in the quality of life of the child
3. Restoration or preservation of immunological function
4. Maximal durable suppression of viral load.

WHO has also developed guidelines for the initiation of ART for the pediatric population based on clinical staging and also the virological test results and CD4 counts or in case this is not available – total lymphocyte count . This is due to the fact that infection in the pediatric age group is complicated by the passive transfer of viral antigen and antibody from the mothers serum.

The specific guidelines are in case **CD4 count is available**,

a. for children less than 18 months –

Virologically positive – treatment is begun for

1.Stage III disease irrespective of CD4 count

. 2.Stage I and II CD4 count less than 20%

Virological test not available – mother HIV positive

Stage III disease CD4 count less than 20%

b. for children more than 18 months –

HIV antibody test positive

1. Stage III disease irrespective of CD4 count

2. Stage I and II CD4 count less than 15%

In case **if CD4 count is not available**, the following criteria is used,

a. For children less than 18 months

Virologically positive – treatment is begun for

1. Stage III disease and AIDS defining illness

2. Stage I and II disease TLC < 2,500

Virological test not available- mother HIV positive

1. Stage III treatment not recommended except AIDS defining disease

b. For children greater than 18 months and HIV antibody test positive

1. Stage III

2. Stage I/II – consider if TLC<1,500.

The various CD4 counts for various age groups for starting therapy:

In less than 12 months CD4 < 20% is equivalent to Absolute CD4 count < 1000/cu mm. In the age group 12-18 months CD4 count <20% corresponds to < 750/cumm. In the age group 1-5 years CD4< 15% corresponds to <500 / cumm and in the age group > 6 years <200/cumm.

What to initiate?

Despite the availability of several drugs in each group of anti-retro viral drugs , WHO has selected 13 drugs for use in regimens to be used in resourse poor countries like India..

Following are the Drugs approved by WHO for ART,

1. Nucleoside reverse transcriptase inhibitors – zidovudine, didanosine, stavudine, lamivudine and abacavir.

2. Nucleotide reverse transcriptase inhibitors – tenofovir disoproxil fumarate.

3. Non-nucleoside reverse transcriptase inhibitors – nevirapine and efavirenz

4. Protease inhibitors- saquinavir, ritonavir, indinavir, nelfinavir and lopinavir/ritonavir.

First line regimen recommended by WHO is Stavudine + Lamivudine + Nevirapine. Stavudine and lamivudine is available as Fixed Dose Combination (FDC).

Second line regimen – recommended by WHO is Zidovudine + Didanosine + nelfinavir.

When to change Anti Retro viral therapy?

Decisions must be based on the following factors:

a) Virologic Considerations

- Less than a minimally acceptable virologic response after eight to 12 weeks of therapy.
- HIV RNA not suppressed to undetectable levels after four to six months of antiretroviral therapy.

b) Immunologic Considerations

- Change in immunologic classification on the worse side.
- A rapid and substantial decrease in absolute CD4+ T cell count

c) Clinical Considerations

Progressive neuro-developmental deterioration.

- Growth failure defined as persistent decline in weight-growth velocity despite adequate nutritional support and without other explanation.

- Disease progression defined as advancement from one pediatric clinical category to another

Dosages and Adverse reactions of the more commonly used anti retroviral agents[5,8]

Drug	Dosage	Adverse Reactions	
	Oral dosages only	**More common**	**Less common but important**
Zidovudine (zdv, azt)	Age: 0-90 days: 2 mg/kg/dose 6th hourly >90 days old: 160mg/m²/dose 8th hourly	Anemia, Granulocytopenia	Hepatotoxicity Myopathy
Lamivudine (3tc)	Age: 0-30 days:2mg/kg/dose 12th hourly >30 days old:4mg/kg/dose 12th hourly	Headache,fatigue, GI intolerance,Rash	Pancreatitis, Peripheral Neuropathy
Stavudine (d4t)	1 mg/kg/dose 12th hourly	GI intolerance Headache	Peripheral Neuropathy, Pancreatitis
Nevirapine (NVP)	0-8 years: 7 mg/kg/dose 12th hourly >8 years: 4mg/kg/dose 12th hourly	Rash,Headache GI intolerance	Hepatotoxicity

PROPHYLAXIS AGAINST OPPORTUNISTIC INFECTIONS:[11,19]

Pneumocystis carinii[16]

Indication: HIV-infected or HIV-indeterminate, infants aged 1-12 mo;

HIV-infected children aged 1-5 yr with CD4+count <500/μL or CD4+ percentage <15%;

HIV-infected children aged 6-12 yr with CD4+ count <200/μL or CD4+percentage <15%.

First Choice**:**Trimethoprim-sulfamethoxazole (TMP-SMZ), 150/750 mg/m²/d in 2 divided doses po t.i.w. on consecutive days

Acceptable alternative dosage schedules:

Single dose po t.i.w. on consecutive days;

2 divided doses po q.d.; 2 divided doses po t.i.w. on alternate days

Mycobacterium tuberculosis [17]

Indication: Mantoux reaction, ≥5mm *or* prior positive mantoux result without treatment; or contact with any case of active tuberculosis regardless of mantoux result

First Choice: Isoniazid 10-15 mg/kg (max 300 mg) po q.d. x 9 mo or 20-30 mg/kg (max 900) mg po b.i.w. x9 months .Rifampin, 10-20 mg/kg (max 600 mg) po q.d. x 4-6 mo

Multidrug-(isoniazid and rifampin) resistant: Choice of drugs requires consultation with public health authorities and depends on susceptibility of isolate from source patient

Mycobacterium avium complex

Indication : For children aged ≥6 yrs, CD4+ count <50/μL;

aged 2-6 yrs CD4+ count <75/μL; aged 1-2 yrs, CD4+ count <500/μL;

aged <1 yr, CD4+ count <750/μL

First Choice: Clarithromycin 7.5 mg/kg (max 500 mg) po b.i.d. , or azithromycin, 20 mg/kg (max 1,200 mg) po q.w.

Varicella zoster virus :

Indication**:** Significant exposure to varicella or shingles with no history of chickenpox or shingles

First Choice: Varicella zoster immune globulin (VZIG), 1 vial (1.25 mL)/10 kg (max 5 vials) im, administered ≤96 hrs after exposure, ideally within 48 hrs

Toxoplasma gondii:

Indication: IgG antibody to Toxoplasma and severe immunosuppression

First Choice: TMP-SMZ, 150/750 mg/m^2/d in 2 divided doses po q.d.

Dapsone (children aged ≥1 mo), 2 mg/kg or 15 mg/m2 (max 25 mg) po q.d. plus pyrimethamine, 1 mg/kg po q.d. plus leucovorin, 5 mg po every 3 days

Invasive bacterial infections:

Indication:Hypogamma-globulinemia (i.e., IgG <400 mg/dL)

First Choice: IVIG (400 mg/kg every 2-4 wks)

Cryptococcus neoformans:

Indication: Severe immunosuppression

First Choice: Fluconazole, 3-6 mg/kg po q.d.

Alternatives**:** Itraconazole, 2-5 mg/kg po every 12-24 h

Clearcut guidelines on when to stop these prophylactic chemotherapeutic agents are yet to emerge.For most diseases, life long therapy with the above mentioned drugs, is warranted if the drug has to be given for prevention of recurrence of disease after the first episode.

PRINCIPLES OF IMMUNIZATION FOR HUMAN IMMUNODEFICIENCY VIRUS-INFECTED CHILDREN:

General principles of immunization in such children include the avoidance of Live Viral and Bacterial vaccines in the severely immunocompromised children.

Other recommendations include:

Infants born to HBsAg- positive mothers should receive Hep B and 0.5 mL hepatitis B immune globulin (HBIG) within 12 hours of birth at separate sites. The second dose is recommended at age 1- 2 months and the third dose at age 6 months. Infants born to mothers whose HBsAg status is unknown should receive Hep B within 12 hours of birth. Maternal blood should be drawn at delivery to determine the mother's HBsAg status; if the HBsAg test is positive, the infant should receive HBIG as soon as possible (no later than age 1 week). All children and adolescents (through age 18 years) who have not been immunized against hepatitis B should begin the series during any visit.

All children should receive four doses of IPV at age 2 months, age 4 months, between ages 6 and 18 months, and between ages 4 and 6 years. Oral poliovirus vaccine should not be administered to HIV infected persons or their household contacts. However there is some controversy on this issue.

Supportive therapy: Even before the antiretroviral agents were available,a significant impact on quality of life & survival of HIV infected children was achieved with intensive supportive therapy. Close attention should be given to nutritional status which is often delicately balanced and may require aggressive pre-emptive intervention to achieve adequate caloric and protein intake . Regular developmental evaluations with necessary physical & speech therapies are a must.

Considerations Regarding the Use of Antiretroviral Drugs by HIV-1-Infected Pregnant Women and Their Infants[6]:

Pregnant women with HIV infection must receive standard clinical, immunologic, and virologic evaluation. The three-part ZDV chemoprophylaxis regimen, initiated after the first trimester, should be recommended for all pregnant women .

HIV-1 infected women receiving antiretroviral therapy in whom pregnancy is identified after the first trimester should continue therapy. ZDV should be a component of the antenatal antiretroviral treatment regimen after the first trimester whenever possible. If therapy is discontinued during the first trimester, all drugs should be stopped and reintroduced simultaneously to avoid the development of drug resistance.

In HIV-Infected Women in Labor Who Have Had No Prior Therapy

Several effective regimens are available . These include the following:

1. single dose nevirapine at the onset of labor followed by a single dose of nevirapine for the newborn at age 48 hours;

2. oral ZDV and 3TC during labor, followed by one week of oral ZDV/3TC for the newborn;

3. intrapartum intravenous ZDV followed by six weeks of ZDV for the newborn; and

4. the two-dose nevirapine regimen combined with intrapartum intravenous ZDV and six week ZDV for the newborn.

In Infants Born to Mothers Who Have Received No Antiretroviral Therapy During Pregnancy or Intrapartum**, ZDV** should be initiated as soon as possible after delivery--preferably within 6-12 hours of birth. Some clinicians may choose to use ZDV in combination with other antiretroviral drugs. The infant should undergo early diagnostic testing so that if HIV-infected, treatment can be initiated as soon as possible.

Exclusive Breast feeding can be given to these children in the first 4-6 months. Then it must be stopped abruptly when top feeding or weaning foods are started, as continuation of Breast milk in addition with other feeds results in higher transmission rates.

Summarizing, a multi disciplinary team approach is desirable for successful management. Necessary components in this approach are methods for monitoring of clinical /nutritional/growth status, appropriate anti-retroviral regimen, monitoring of therapy, prophylaxis against opportunistic infection, appropriate immunization, adequate supportive care and counseling as and when required. Love, affection and optimism from all the caregivers can do wonders to the mental health of HIV infected child. However, universal HIV testing with patient notification and reduction of perinatal transmission holds the key to phenomenal success in the management of HIV infected children.

REVIEW OF LITERATURE

Costella et al [46] compared the total lymphocyte count to CD4 lymphocyte cells count in 839 adult patients with HIV in Thailand. Their study found out a correlation of 0.67 between both these factors. It also showed a higher correlation (0.72) for the female sex than in males (0.68) .Subset analysis by WHO clinical stages revealed a higher correlation (0.73) in the more severe disease groups (II & III) than in Group I. Their study was one of the few studies that combined correlation coefficients for hemoglobin as well as total lymphocyte counts and compare to them to CD4 + T lymphocyte cell count. It showed that such an approach was more accurate in predicting CD4 + T lymphocyte cells.

Mahajan et al[45], from Los Angles conducted a study that compared total lymphocyte count with CD4 + T lymphocyte cell count following the initiation of HAART. Their study showed that decrease in the CD4 + T lymphocyte cell counts following HAART was closely paralleled by a corresponding and highly correlative decrease in total lymphocyte count also. This change however continued only upto two years of HAART therapy. Though this was one of the few studies that tried to analyse the correlation following HAART. This study was also hampered by the small sample size (126 patients).

Elna Van der ryst et al and Marinde et al [28] measured CD4 counts and total lymphocyte counts of 2777 HIV seropositive patient visiting the

immunology clinic at pelonomi hospital in South Africa between 1991 –1997, which accounted for 3237 observations of CD4 counts, CD4%, Total lymphocyte counts and CD8 T cells. Spearman rank correlation was calculated between total lymphocyte counts and CD4 counts. There was a high degree of correlation (R=0.7060) between CD4 counts and total lymphocyte counts.

Kumarasamy et al and Mahajan[34] et al assessed the correlation between CD4 counts and total lymphocyte counts in HIV seropositve patients attending an HIV / AIDS clinic at Chennai. Positive predictive value negative predictive value, and sensitivity and specificity of various total lymphocyte cut off were computed for CD4 counts < 200 cells /mm3 and < 350 cells / mm3. High degree of correlation (0.744) was noted between 650 paired CD4 and total lymphocyte counts. Total lymphocyte counts < 1400 cells had 76% positive predictive value and 86% negative predictive value and was 73% sensitive and 88% specific for CD4 counts <200 cells /mm3. Total lymphocyte count <1700 cells /mm3 had 86% PPV, 69% NPV and was 70% sensitive 86% specific for CD4 counts <350 cells /mm3. This is one of the very few studies done in Indian Population. They concluded that total lymphocyte could serve as low cost tool for determining both the patient risk of opportunistic infection and when to initiate prophylaxis in resource constrained settings.

E.J. Beck et al, and Kupek et al[38]assessed the correlation between total lymphocyte count, an absolute CD4 counts in HIV seropositive patient managed at St.Marys Hospital , London. Spearman partial rank correlation

was calculated between total lymphocyte count and absolute CD4 counts, CD4% stratified by stage of HIV infection. There was a high degree of correlation between 1534, paired absolute lymphocyte counts and CD4 lymphocyte counts. When analyzed by stage of HIV infection the correlation increased from (R=0.64) for asymptomatic patients to(R= 0.72) for patients with symptomatic non AIDS HIV and to (R=0.73) for AIDS patients. The high degree of correlation between total lymphocyte count and CD4 lymphocyte counts especially for symptomatic AIDS patients demonstrates the suitability of total lymphocyte counts as a marker of immune status especially where CD4 counts are not available.

Post et al[39] compared total lymphocyte counts and CD4 lymphocyte counts of 831 HIV / AIDS patients from Cape Town, South Africa as predictors of developing AIDS /death. They concluded that a total lymphocyte count < 1200/mm^3 and a CD4 count <200 cells /mm^3 were equal predictive of disease progression and that a total lymphocyte count of 1200/mm^3 could be used as cutoff for initiating cotrimoxazole prophylaxis.

Stebbing et al and Sawleshwarhar et al studied the utility of total lymphocyte count in place of CD4 count to predict the development of AIDS defining opportunistic infection.

CD4 lymphocyte count and total lymphocyte count were recorded in those people who had a first episode of an AIDS defining opportunistic infection . Pearson correlation coefficient was 0.70 between these variables with a P value of less than 0.001 which was stastically significant. Patients

with total lymphocyte counts of 1000 –1500 cells/mm^3 were estimated to be at 40% increased risk of developing AIDS defining opportunistic infection , whereas patient with CD4 count of 150-200 cells /mm3 were at 34% increased risk of developing an AIDS defining opportunistic infection. They concluded that total lymphocyte count can be used as reliable predictors of AIDS defining opportunistic infection in HIV infected people and as such total lymphocyte count can also be used to facilitate decision about timing of HAART and. AIDS defining opportunistic infection prophylaxis.

Motasim Badri et al and Robinwood et al[40] assessed the usefulness of total lymphocyte count in monitoring highly active anti retroviral therapy in 266 patients attending HIV clinic / research unit at somerset hospital capetown.CD4 count and total lymphocyte counts were measured from baseline at 4,8,12,48 weeks after initiation of highly active antiretroviral therapy. The median increase in total lymphocyte counts was 30, 52,139 and 219 cells X 10^6 / l, median increase in CD4 count was 8,48,88 and 145 cells X 10^6 /l.

There was a high degree of correlation (R=0.611) between all pairs of CD4 count and total lymphocyte counts. Total lymphocyte count correlated well with changes in CD4 cell count. They concluded total lymphocyte may have a role in inexpensive monitoring of immunological response to HAART resource constrained settings.

STUDY JUSTIFICATION

More than 90% of HIV infected individuals reside in resource-constrained countries[1]. In resource-rich settings, CD4 cell count and HIV-1 RNA concentration are measured regularly in HIV-infected individuals to guide decisions about opportunistic infection prophylaxis ,antiretroviral therapy initiation and maintenance. In some settings, the cost of monitoring HIV-1 treatment now exceeds the cost of antiretroviral drugs[33]. As initiatives to provide antiretroviral therapy in resource-poor settings are being undertaken, it is important to evaluate alternative assays that could guide treatment decisions. Current guidelines recommend that decisions involved in initiating and changing antiretroviral therapy be guided by the immune category of children, based on their CD4 cell counts. Where CD4 count testing is not available or too expensive for routine use, the WHO recommends the use of total lymphocyte count (TLC) to monitor the immune response to HAART. TLC is an inexpensive and widely available laboratory parameter. TLC is easily obtained from the routine complete blood count (CBC) with differential by multiplying percentage lymphocytes by white blood cell count. In southern India for example, the cost of a single TLC from a CBC is less than 100 rupees while a single CD4 count by flow cytometry is approximately 1500 rupees. There are no of studies examining the correlation between total lymphocyte counts and CD4 counts in HIV +ve adult patients.There is no published data examining the correlation between total lymphocyte counts and CD4 counts in HIV seropositive pediatric patients. In our study we have examined the correlation between total lymphocyte counts and CD4 counts in HIV infected children between 2 to 12 years of age.

AIM OF THE STUDY

PRIMARY OBJECTIVE

To study the correlation between total lymphocyte counts and CD4 lymphocyte counts of children belonging to stages I , II and III of WHO staging for AIDS.

SECONDARY OBJECTIVE

To establish arbitrary cut off values of total lymphocyte counts that correspond to CD4 counts less than 500/mm^3 in the age group 2 – 5 years and less than 200/ mm^3 in 6 – 12 years age group.

MATERIALS AND METHODS

Study Design :

Descriptive study

Study period :

07/07/2004 to 15/08/2005

Study place :

Institute of Child Health &Hospital for Children ,Chennai

Study population :

All children between 2 and 12 years diagnosed as having HIV positivity, by means of repeatedly positive ELISA, who were admitted in, or referred to, our Institute.

SAMPLE SIZE

With a α - error of 0.05 and a power of the study corresponding to 80% and with previous study of Kumarasamy et al., based on adults showing a correlation of 0.744 and with an expected correction 0.70, sample size calculated statistically for this study to show meaningful results was 150.

MANEUVER

A thorough history and clinical examination was performed in all these children, the details of which were noted in a predesigned proforma. These

included age, sex, risk factors for HIV infection, time of onset of symptoms, duration of symptoms, examination findings with special reference to growth failure, fever, hepato-megaly, splenomegaly, lymphadenopathy, parotitis, diarrhea, secondary infectious diseases and other associated diseases. . Routine laboratory investigations including a complete blood count, X-ray chest, Mantoux test and urine and stool examination was done in all children. Specific investigations which included cerebrospinal fluid (CSF) examination, gastric lavage for acid fast bacilli (AFB), fine needle aspiration cytology (FNAC), blood culture, relevant imaging studies were done when indicated.Their parents were screened for HIV positivity by means of ELISA. Siblings were also screened for HIV positivity.

CD4 counts and Total Lymphocyte Counts were measured on the same blood sample taken in EDTA coated tubes. The total and differential cell counts were determined using an automated hematology analyzer (Abx, France) and TLC was derived from the CBC by multiplying lymphocyte percentage by the white blood cell count. CD4%, was measured in whole blood using a 3 colour panel purchased from Beckman coulter,USA, using a standard protocol.The monoclonal anti CD4 antibody was directly conjugated with RD1 (phycoerythrin), anti CD8 with fluorescin isothiocyanate and anti CD3 with PC5. The cells were analysed on a Beckman coulter Epics Altra flowcytometer cum sorter using EXPO32 multiCOMP software. Data was collected for 10,000 cells for each tube and displayed as dot plots. Dual positive CD3+ CD4+ and CD3+ Cd8+ cell % were measured and the absolute CD4 count was derived by multiplying CD4% and TLC. They were given supportive treatment mainly

consisting of PCP prophylaxis, nutritional support, control of other infections, anti-tuberculous therapy wherever applicable.The option of Anti Retro-Viral therapy was discussed with the caregivers. .

STATISTICAL ANALYSIS

Correlation between CD4 count and total lymphocyte count was assessed by computation of spearman rank order correlation for all paired count

Sensitivity, specifity, positive predictive value and negative predictive value of various total lymphocyte counts ranges were calculated for CD4 count < 200 cell/mm^3 in the age group 6-12 and CD4 count < 500 cells /mm^3 in the age group 2- 6 years.

RESULTS

150 patients were included in the original study group based on the statistically derived sample size. Since 2 of these patients had significantly elevated TLC and CD4 cell counts that skewed the ananlysis of data for correlation, these two samples were excluded from the study. The remaining 148 pairs of values were taken into consideration for the calculation of spearman rank correlation coefficients between TLC and CD4 cell counts.

Table:1

Demographic Details of the Study Population

Demographic data		**No. of children n**	**No.of children in %**
Age group (years)	2-5	91	61.5
	6-12	57	38.5
Sex distribution	M	99	66.9
	F	49	33.1
Parental HIV status	Mother +	122	81.3
	Mother -	4	2.1
	Mother status NK	25	16.5
	Father +	94	62.5
	Father -	16	10.4
	Father status NK	41	27.1

Table : 2

Mode of Transmission of HIV in Study Population

Mode of transmission	No. of children(n)	No . of children in %
MTCT	131	87.3
Blood	1	.0066
Not known	18	12

87.3% of HIV infected children were infected by vertical transmission , only 1 case of confirmed HIV was discovered to have been transmitted by blood transfusion . In 12% of the children mode of transmission could not be found because parents could not be screened and some of these children were from orphanages

Table - 3

Clinical categorization of Study Population according to WHO STAGING

WHO staging	Frequency	Percent
I	15	10.1
II	92	62.2
III	41	27.7

The study population was categorized in to groups according WHO clinical staging. Majority of these children belonged to stage II (62%) and 27% of the children belonged to stage III.

Table - 4

Immune categorization of Study Population

Immune category	No. of children by CD4+ T cell count	% of children by CD4+ T cell count	No. of children by CD4+ T cell %	% of children by CD4+ T cell %
No evidence of immunosuppresion	21	14.2	1	.7
Evidence of moderate immunosuppresion	96	64.9	82	55.4
Evidence of severe immunosuppresion	31	20.9	65	43.9

Immune categorization was done on these children based on CD4 lymphocyte count and CD4 lymphocyte percentages. 64% of the children and 55% of the children were moderately immune suppressed based on their CD4 counts and CD4 percentages respectively.

Table - 5

Spearman rank correlation coefficient between total lymphocyte count and CD4 count in the entire study population

	Total lymphocyte count	**CD4 count**
Total lymphocyte count	1.000	.797
CD4 count	.797	1.000

The spearman correlation coefficient for CD4 to total lymphocyte was 0.797 in the study population

Table - 6

Spearman rank correlation coefficient between total lymphocyte count and CD4 count in groups stratified based on who clinical stagings

WHO Staging		CD4 count	Total lymphocyte count
I	CD4 count	1.000	.887
	Total lymphocyte count	.887	1.000
II	CD4 count	1.000	.843
	Total lymphocyte count	.843	1.000
III	CD4 count	1.000	.712
	Total lymphocyte count	.712	1.000

Spearman rank correlation was analyzed in children belonging to different stages of WHO staging of AIDS. The correlation coefficient was 0.887 in WHO stage I and 0.712 in WHO stage III.

Table - 7

Spearman rank correlation coefficient between total lymphocyte count and CD4 count in groups stratified according to age

Age Group		CD4 Count	Total Lymphocyte Counts
2-5 years	CD4 Count	1.000	.791
	Total Lymphocyte counts	.791	1.000
6-12 years	CD4 Count	1.000	.823
	Total Lymphocyte counts	.823	1.000

The correlation coefficient was 0.791 in the age group 2-5 years, 0.823 in the age group 6-12 years.

Table - 8

Positive predictive value (PPV), negative predictive value (NPV), and sensitivity and specificity of various TLC cutoffs for CD4 counts <500cell /mm^3 in children between 2-5 years

TLC cutoff	Sensitivity	Specificity	PPV	NPV
<2000	35	100	100	87
<2500	47	100	100	89
<3000	65	100	100	93
<3500	**82**	**78**	**48**	**95**
<4000	82	75	38	96
<4500	94	69	36	98
<5000	94	53	27	98
<5500	100	37	23	100

A total lymphocyte count value of < 3500 cells/ mm^3 had a sensitivity of 82% and a specificity of 78% for a correlation with a CD4 lymphocyte count of < 500 cells/mm^3.

Table - 9

Positive predictive value (PPV), negative predictive value (NPV), and sensitivity and specificity of various TLC cutoffs for CD4 counts <200cell /mm^3 in children between 6-12 years

TLC cutoff	Sensitivity	Specificity	PPV	NPV
<1000	36	98	83	82
<1500	**86**	**86**	**67**	**95**
<2000	86	55	44	90
<2500	93	54	39	96
<3000	93	35	32	94
<3500	100	14	27	100

A total lymphocyte count value of < 1500 cells/ mm^3 had a sensitivity and specificity of 86% for a correlation with a CD4 count of < 200 cells/ mm^3

DISCUSSION

CD4 lymphocyte are the principal targets of HIV and progressive decline of these cells over time takes place in HIV infection. CD4 T lymphocyte count is a useful indicator of disease progression and is widely used to determine points at which prophylaxis against opportunistic infections should be instituted and also for monitoring response to HAART. However owing to the high cost and need for specialized equipment and wide spread and routine use of CD4 count in the management of HIV infection has not been possible. There is a need for simpler ,cheaper surrogate markers to monitor HIV disease progression especially in resource limited settings such as ours. WHO guidelines now recommends that immune categorization and initiation of HARRT be based on clinical indicators in association with basic laboratory tests like total lymphocyte counts and hemoglobin. Though reports suggesting that total lymphocyte counts is a reliable surrogate marker of immune status in adults no such study is available where children with HIV are concerned.

This study aimed at finding out any correlation that exists between total lymphocyte counts and CD4 T cell counts if any and also strength of any such correlation.

Previous studies have also attempted a similar correlation between total lymphocyte counts and CD4 lymphocyte counts in adults.

The correlation obtained between TLC and CD4 counts in the studies as follows.

Beck et al[38] from Newcastle general hospital in London found an correlation of 0.76 between CD4 and total lymphocyte counts in the whole group of patients.

Rysst et al[42] from Bloem fontein in South Africa observed a correlation of 0.704 between total lymphocyte counts and CD4 counts.

Kumaraswamy et al[34] discovered a correlation of 0.744 in 650 samples.This was an Indian study in adults.

Motasim Badri et al [40]in his study of 26 patients in somerset should a correlation of 0.61.

Speech et al in a large study involving 1451 patients between the ages 24-62 years found a correlation of 0.72.

All the above studies have been conducted in adults. The situation in children regarding such a correlation in more complex and there have been very few studies conducted in children in this aspect.

Unpublished data received by personnel communication from Dr.Sowmya Swaminathan in Chennai should a correlation of 0.71%. This has been the only pediatric study that has been Conducted so far in India.

In our study the correlation obtained was 0.797. This has been the highest correlation obtained so far in a study in children conducted in India.

The correlation coefficient is comparable with those of various adult studies indicating that that total lymphocyte counts can be used a surrogate marker of immune status of HIV affected children. Strength of correlation is sufficient to allow a wide confidence interval when predicting CD4 lymphocyte counts based on total lymphocyte counts.

We also calculated the correlation coefficient for different age groups since there is a variability in the total lymphocyte counts and CD4 counts especially in younger age groups .Composition of the various lymphocyte subgroup undergoes significant changes as age advances.

A previous study should be correlation coefficient of 0.67 in 1-5 years ages and 0.74 in 6-12 years. In our study we obtained a correlation coefficient of 0.791 in the age 2-5 years age group and 0.823 in the 6-12 years age group. The value was similar to that obtained in the total group of patients indicating that even in the younger age group total lymphocyte counts can be used as surrogate markers. Correlation coefficient were also obtained in the various subgroups of patients based on WHO staging .Though previous studies in adults showed that the degree of correlation measured increased as the severity of disease progressed (Beck et al[38], Rysst et al[42]). Our study showed that the degree of correlation weakened as severity of disease progressed but not to a very significant extent (stage I-R =0.887 ; Stage II R=0.843 Stage III R=0.712).

Though we had a smaller sample size since this was one of the few studies done in children regarding this aspect, we also tried to establish

various cutoff values of total lymphocyte counts for severe stages of immunosuppression in the age group of 2-5 years and 6-12 ages. These cutoff value of total lymphocyte count correspond to CD4 counts of 200/mm^3 and 500/mm^3. It is these values of CD4 count which are considered for initiation of HAART and well as prophylaxis for various Oppurtunistic Infection. A cut off value total lymphocyte count that corresponds closely with these value of CD4 count would help immensely in practical management of HIV infected children . Similar cutoff values were established in previous adult studies. One of these studies done by Kumarasamy et al[34] suggested that total lymphocyte count of less than 1400 /mm^3 corresponds to a CD4 lymphocyte count of less than 200/ mm^3 and a total lymphocyte count less than 1700/ mm^3 correspond to CD4 count less than 350/ mm^3This study also suggested that selection of appropriate total lymphocyte cutoff should be made only on a regional basis taking in to consideration such as incidence of OI ,local antimicrobial resistance pattern and available of antimicrobials .we derived a total lymphocyte count cutoff for 1- 5 years and 6-12 years respectively .

A total lymphocyte of <3500/ mm^3 correlated with CD4 count < 500/ mm^3in the 2-5 years age group and this value had an sensitivity of 82% and specificity of 78%. Though lower values such as total lymphocyte count <2500/mm^3 had a specificity of 100% they were highly insensitive with sensitivities ranging to from 35- 47% where as with higher values such as <4000 /mm^3 the specificity dropped drastically ,the sensitivity reached 100% .Similarly we obtained a value of total lymphocyte count<1500 as being 86% sensitive as well as specific for CD4 <200 / mm^3 in children between 6-12

years. These values are arbitrary cutoff values that have been derived based on the WHO definition of severe immunsuppresion in children and as stated by the previous studies these value also need modification based on locoregional sensitivity pattern of micro organism and existing level of OI.

Our study has some limitation as we included data from all available children regardless of there clinical status, presence of tuberculosis and other opportunistic infections. Most of these children were malnourished and were on therapy for other infections. However none were receiving antiretroviral therapy. Our aim was to be as inclusive as possible in order to get such a result that would be applicable in a real world clinical setting such as ours, where HAART and other aggressive modes of therapy are not yet freely available due to economic and social constraints.

It would be ideal to study a large number of patients using these data available now as a pilot study. This can be of great use in the resource limited settings like ours from therapeutic point of view.

Other surrogate to marker for which evaluation is ongoing in various trails includes hemoglobin. Combined correlation using both total lymphocyte count and hemoglobin can increase the predictive value of estimating CD4 count based on these surrogate markers.

CONCLUSION

Correlation coefficient obtained for the whole group of patients between total lymphocyte count and CD4 count was 0.812.

Correlation was strong in all age groups but strongest in the in the 6-12 years age group. Strength of correlation is comparable with that obtained in previous adult studies.

Correlation coefficient was also strong in the various subgroups of patients based on WHO staging (stage I-R =0.887 ; Stage II R=0.843; Stage III R=0.712).

We have shown that a TLC of 3500 cells / mm^3 in children, 1-5 years of age and 1500 cells / mm^3 in those aged 6 - 12 years correlates with a CD4 count of <500 cells / mm^3 and 200 cells / mm^3 respectively, with a sensitivity and specificity between 78 - 86%.

TLC can be used as a surrogate marker of immune status in resource poor settings such as ours instead of CD4 lymphocyte counts.

Surrogate markers such as hemoglobin which is another easily available investigation, in most health care centre, even in resource restricted settings, need to be evaluated further.

A correlation coefficient combining the total lymphocyte count and hemoglobin can be more useful than either of the two, both for predicting CD4 lymphocyte count and also the cut off values for initiating HAART.

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45. Changes in Total Lymphocyte Count (TLC) as a Surrogate for Changes in CD4 Count following Initiation of HAART: Implications for Monitoring in Resource-Limited Settings Anish p. Mahajan1*, Joseph w. Hogan2, brad snyder2, n. Kumarasamy3, kalindi mehta1, Suniti solomon3, Charles c. J. Carpenter1, Kenneth h. Mayer1, and timothy p. Flanigan1.

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47. Predictors of Low CD4 Count in Resource-Limited Settings: Based on an Antiretroviral-Naive Heterosexual Thai Population**.** JAIDS Journal of Acquired Immune Deficiency Syndromes. 39(2):242-248, June 1, 2005.

ANNEXURE - I

PROFORMA

Name :

Age :

Sex :

Mode of Delivery
& Perinatal Details :

Parental HIV Status :

Age of Presentation :

Clinical Features : Duration of Symptoms

Failure to Thrive

Recurrent Diarrhea

Prolonged Fever

Recurrent respiratory tract infections.

General Examination :

Systemic Examination

CVS :

RS :

ABDOMEN :

CENTRAL NERVOUS SYSTEM

LABORATARY INVESTIGATIONS :

COMPLETE BLOOD COUNT &
PERIPHERAL SMEAR :

URINE ROUTINE EXAMINATION :

CHEST X-RAY :

MANTOUX :

SPUTUM FOR AFB :

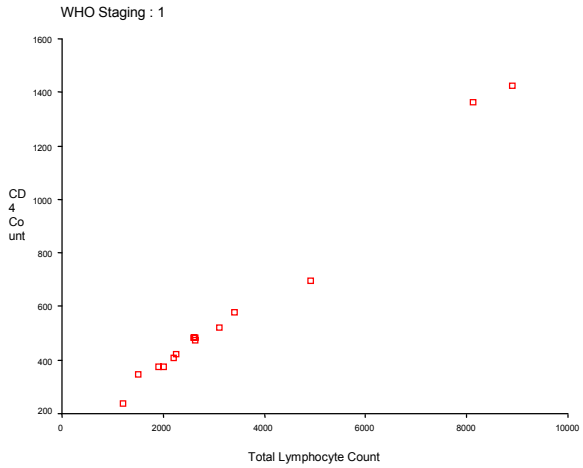
CD4 COUNTS :

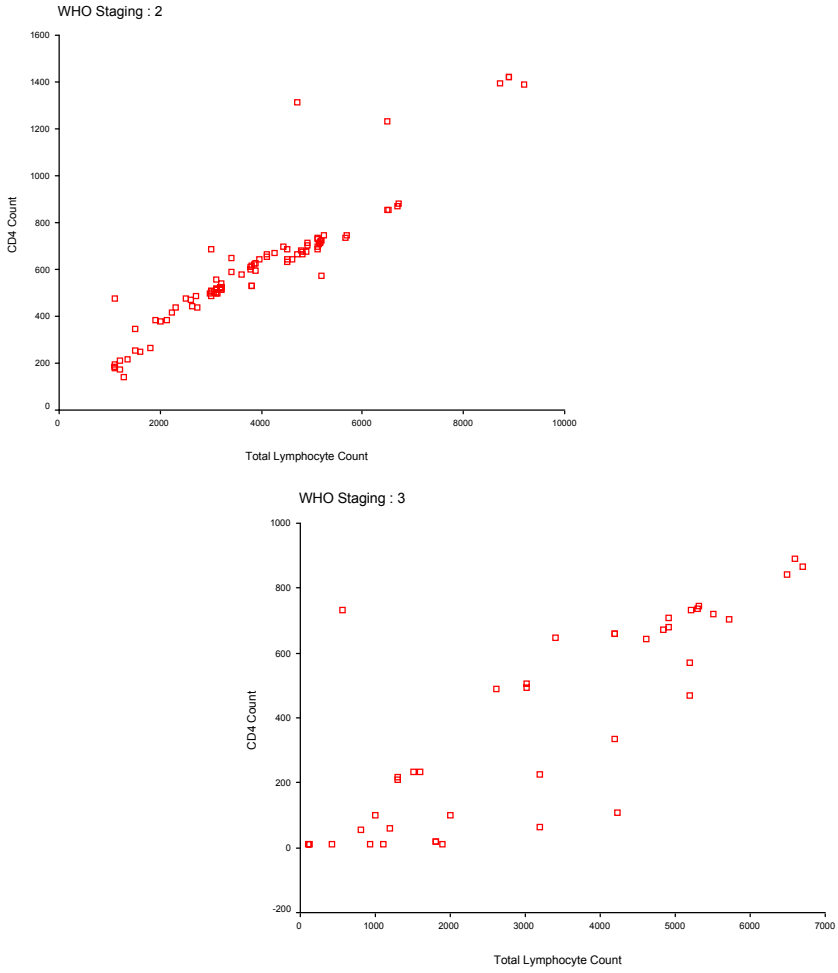
CD4 PERCENTAGE :

TOTAL LYMPHOCYTE COUNTS :

ANNEXURE - II

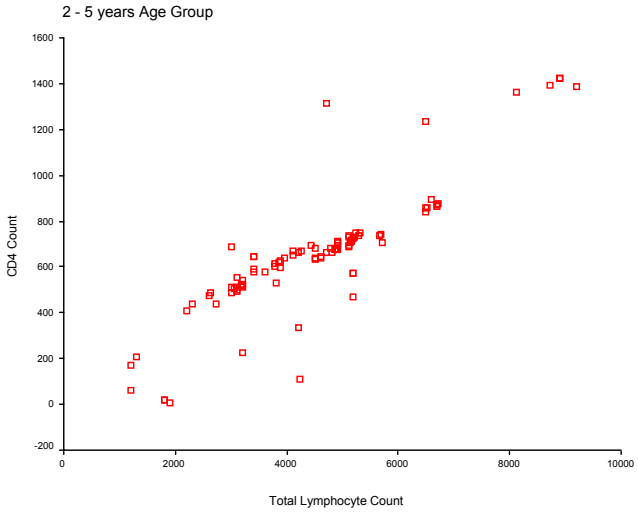
GRAPH SHOWING CORRELATION BETWEEN CD4 LYMPHOCYTE COUNTS AND TOTAL LYMPHOCYTE COUNTS IN STAGES I, II, III OF WHO STAGING FOR AIDS

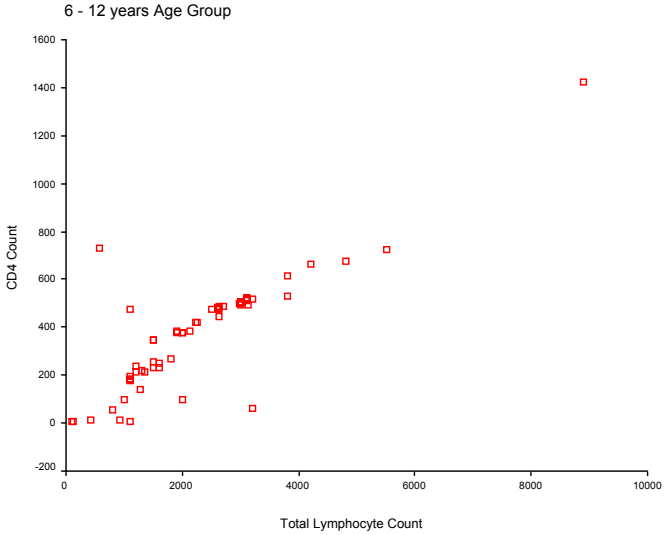




ANNEXURE - III

GRAPH SHOWING CORRELATION BETWEEN CD4 LYMPHOCYTE COUNTS AND TOTAL LYMPHOCYTE COUNTS N AGE GROUP 2 TO 6 AND 6 TO 12 YEARS


2 - 5 years Age Group
CD4 Count
Total Lymphocyte Count
1600
1400
1200
1000
800
600
400
200
0
-200
0
2000
4000
6000
8000
10000


6 - 12 years Age Group
CD4 Count
Total Lymphocyte Count
1600
1400
1200
1000
800
600
400
200
0
-200
0
2000
4000
6000
8000
10000